Gene expression profiling in ANCA-vasculitis renal biopsies

Marcia Friedman, MD
Northwest Rheumatism Society Meeting
OHSU Fellow Research Presentation
April 29th 2017
Disclosures

• I have no disclosures
Background

• Significant recent advancements in AAV pathophysiology highlighting the role of netosis, pathogenicity of ANCAs, and the alternative complement system as an amplification pathway in active disease.

• Little is known about the mechanism of disease damage at the end-organ level

• Gene expression analysis is an important tool to identify precise disease-specific treatment targets

• This project aims to apply this analysis technique to the study of the ANCA-associated vasculitis (AAV) kidney.
Gene expression profiling in AAV

• Whole blood, leukocytes, nasal brushings, orbital tissue, and renal tissues

1. RAVE study patients: **whole blood** during active disease and remission:
   • 179 genes predominantly including granulocyte related genes, MPO, and PR3 - paid particular attention to 11 genes selected to represent the spectrum of neutrophil granular proteins.
   • Compared responders vs non-responders; high levels of granulocyte gene expression predicted lower likelihood of treatment response.

*Arthritis Rheumatol.* 2015 Jul;67(7):1922-32
Gene expression profiling in AAV

2. Nasal turbinate brushings from
   - 32 GPA patients (10 active, 13 prior sinus disease, 9 no hx of nasal disease)
   - 12 healthy, 15 sarcoid, 8 allergic rhinitis.
   - Genes related to granulocyte and agranulocyte migration/adhesion, epithelial barrier integrity, IL10 signaling, and TREM1 signaling
   - PR3 and MPO genes were not differentially expressed
   - Differences between active/quiet GPA patients: 18 genes differentially expressed including genes involved in cell adhesion and matricellular proteins

Arthritis Rheumatol. 2015 May; 67(8): 2233–2239.
Gene expression profiling in AAV

3. **Leukocytes** in AAV, SLE, and RA compared to healthy donors.
   - Differential expression in AAV, SLE, and RA
   - AAV genes involved in innate immunity, inflammatory processes, and stress/wounding genes. (TLR genes, B-cell lymphoma 6 gene, and IL-1 receptions I/II genes)
4. AAV Renal Gene Expression: 2016 ACR Abstracts

- Eddy et al. Inflammatory pathways as **shared molecular targets** across AAV and nephrotic syndrome:
  - Samples from NEPTUNE cohort and European Renal cDNA Bank
  - Searching for shared therapeutic targets of nephrotic syndrome: Tec Kinase, IL-8 signaling, TNF, IFNG, TGFB1, and NFkappaB

- Grayson et al. **Immunometabolism in ANCA-associated GN**:
  - Samples from the European Renal cDNA Bank and other groups
  - Upregulation of pentose pathways (G6PD) and glycolysis regulatory genes.
  - Downregulation of Krebs cycle, glutaminolysis, and fatty acid oxidation genes.
  - Indicated metabolism of glucose as a novel treatment approach

- Both studies lack clinical data
4. AAV Renal Gene Expression

• 28 MPO-AAV Renal biopsies: evaluating genes expressed in ANCA-vasculitis **tubulointerstitial injury**
• Tubulointerstitial injury severity correlated with IL-1beta expression.
• Also identified TLR4 and NLRP3 correlating with severity of injury

Gene expression profiling can distinguish a subset of orbital pseudotumor due to GPA from sarcoid, thyroid eye disease, and controls.

A subset of idiopathic orbital inflammatory disease clusters with GPA suggesting this as a possible diagnostic tool in identifying the underlying disease in idiopathic cases.
## Preferentially expressed genes across studies

<table>
<thead>
<tr>
<th>Tissue</th>
<th>MMP9</th>
<th>IL-7R</th>
<th>CD64</th>
<th>IL-1</th>
<th>TCR1 beta</th>
<th>SLC11</th>
<th>TLR2</th>
<th>TREML</th>
<th>TLR-8</th>
<th>Defensin</th>
<th>SERPINA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>IL-1R antagonist</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal brushings</td>
<td></td>
<td></td>
<td></td>
<td>IL-1 beta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DEF4A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IL-1R type II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orbit</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>IL-1R antagonist</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Specific Aims:

1. Test the hypothesis that a characteristic gene expression profile distinguishes ANCA-associated vasculitis from other causes of nephritis.

2. Determine whether subsets of ANCA-associated vasculitis have distinguishable gene expression profiles at a tissue level, and explore their clinical significance.

3. Compare gene expression profiles across tissues affected by ANCA-associated vasculitis to identify commonly preserved patterns, in an attempt to identify novel treatment targets.
Study design and methods: samples

- Renal biopsy samples that have been collected from AAV patients
- Normal controls: nephrectomies of RCC, histologically normal portion of the kidney to be confirmed by renal pathology.
- Inflammatory control group: Interstitial nephritis samples
- Inflammatory glomerular control group: Lupus nephritis samples
- All biopsy samples must be <3 years old, older sample RNA is likely to be too degraded.
Study design and methods: clinical data

• Retrospective chart review:
  • Demographic data
  • Clinical dx (GPA/MPA)
  • Serology (p-ANCA, c-ANCA, MPO, PR3)
  • Treatment prior to biopsy
  • Treatment after biopsy
  • Treatment response/outcome
  • Other organ involvement

• Pathology data: number of glomeruli involved, histopathologic description
Study design and methods: data analysis

<table>
<thead>
<tr>
<th>Power</th>
<th>N</th>
<th>Fold change</th>
<th>K</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8</td>
<td>10</td>
<td>2.43</td>
<td>1%</td>
<td>0.05</td>
</tr>
<tr>
<td>0.8</td>
<td>10</td>
<td>2.01</td>
<td>10%</td>
<td>0.05</td>
</tr>
<tr>
<td>0.8</td>
<td>10</td>
<td>1.88</td>
<td>20%</td>
<td>0.05</td>
</tr>
<tr>
<td>0.8</td>
<td>20</td>
<td>1.76</td>
<td>1%</td>
<td>0.05</td>
</tr>
<tr>
<td>0.8</td>
<td>20</td>
<td>1.59</td>
<td>10%</td>
<td>0.05</td>
</tr>
<tr>
<td>0.8</td>
<td>20</td>
<td>1.53</td>
<td>20%</td>
<td>0.05</td>
</tr>
<tr>
<td>0.8</td>
<td>30</td>
<td>1.57</td>
<td>1%</td>
<td>0.05</td>
</tr>
<tr>
<td>0.8</td>
<td>30</td>
<td>1.45</td>
<td>10%</td>
<td>0.05</td>
</tr>
<tr>
<td>0.8</td>
<td>30</td>
<td>1.40</td>
<td>20%</td>
<td>0.05</td>
</tr>
</tbody>
</table>

N: number of samples per group
K: proportion of truly differentially expressed probe sets

- 20 ANCA vasculitis samples gives 80% power to detect a 1.6 fold difference in gene expression if at least 10% of probes are differentially expressed.

- Aiming for:
  - 20 ANCA
  - 20 healthy control
  - 10 lupus
  - 10 interstitial nephritis
Statistics

• Training set: 70% of samples will be used to develop a training set of genes.
• Test/validation set: remaining 30% of samples will be used to test and validate that set of genes.
• Principal coordinate analysis (PCA)
  • Visualizing similarity or dissimilarity in a large complex data group
  • All gene probes that show a significant difference between at least one disease group and the control group are used to create a 3D plot
  • Each patient sample is represented on that 3D graph where proximity indicates greater similarity.
Statistics

• Unsupervised hierarchical clustering analysis using a dendrogram
  • First to see if AAV groups differently other forms of nephritis
  • Second to distinguish MPO and PR3 subsets.

• Gene Set Enrichment Analysis program
  • Identifies sets of genes with enriched expression which lends insight into the affected pathways.
  • Data used to generate heat maps
1. Do two year old FFPE renal biopsies contain adequate RNA for analysis?
   • Pilot study of three 2-year-old renal biopsy samples sent for RNA extraction.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Delivery Date</th>
<th>Delivery State</th>
<th>Section/ block age</th>
<th>Elution Volume (µl)</th>
<th>A260</th>
<th>A280</th>
<th>260/280</th>
<th>260/230</th>
<th>Constant</th>
<th>RNA Concentration (ng/µl)</th>
<th>RNA Yield (ng)</th>
<th>RIN</th>
<th>Dil. Factor</th>
<th>RNA(µl)</th>
<th>Water (µl)</th>
<th>Estimated conc (ng/µl)*</th>
<th>Estimated Yield (ng)</th>
<th>DV200</th>
</tr>
</thead>
<tbody>
<tr>
<td>P001</td>
<td>1/24/17</td>
<td>FFPE ambient</td>
<td>10 micron section</td>
<td>20</td>
<td>2.4</td>
<td>1.3</td>
<td>1.9</td>
<td>1.6</td>
<td>40.0</td>
<td>95.7</td>
<td>1914.4</td>
<td>1.8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>87.0</td>
<td>1740</td>
</tr>
<tr>
<td>P002</td>
<td>1/24/17</td>
<td>FFPE ambient</td>
<td>10 micron section</td>
<td>20</td>
<td>0.8</td>
<td>0.4</td>
<td>1.9</td>
<td>1.3</td>
<td>40.0</td>
<td>33.3</td>
<td>665.6</td>
<td>2.8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>32.0</td>
<td>640</td>
</tr>
<tr>
<td>P003</td>
<td>1/24/17</td>
<td>FFPE ambient</td>
<td>10 micron section</td>
<td>20</td>
<td>1.4</td>
<td>0.7</td>
<td>2.0</td>
<td>1.9</td>
<td>40.0</td>
<td>55.1</td>
<td>1102.4</td>
<td>3.3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>57.0</td>
<td>1140</td>
</tr>
</tbody>
</table>
Preliminary Data

- AAV Samples: total of 49 samples identified
  - 3 excluded because of genetic opt out, leaving 46 samples
- IRB amendment pending to allow us to obtain outside clinical data
- One renal biopsy sample where the patient has either SLE or AAV
- One biopsy sample with interstitial nephritis and positive ANCAs

<table>
<thead>
<tr>
<th>AAV Biopsy Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of samples</td>
</tr>
<tr>
<td>&lt;2 years old</td>
</tr>
<tr>
<td>2-3 years old</td>
</tr>
</tbody>
</table>
Preliminary Data

• Interstitial nephritis samples: heterogeneous group
  • Drug induced interstitial nephritis
  • Sarcoidosis
  • Idiopathic AIN
  • Tubulo-interstitial nephritis and uveitis

• Lupus nephritis samples (pending)

• Control samples:
  • Clear cell RCC nephrectomies
  • Other cancers: oncocytoma, urothelial carcinoma
  • Reflux nephrectomy
Strengths of this study and future directions

• Previous studies of the kidney have only looked at specific pathways and did not have clinical correlates.

• Gathering data in a hypothesis free manner, then using this data to test hypotheses.

• Kidney is a commonly involved organ with significant morbidity. May tell us more about mechanism of end organ damage than peripheral plasma/serum evaluation.

• Looking forward: comparing results with data from other tissues to identify commonly preserved pathways of disease--ultimately to find novel treatment targets.